Spatially resolved, scattered light using a high numerical aperture microscope: a new method to determine the signatures of normal and cancerous cells

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Extended Abstract

Recent measurements by Perelman et al. have shown that it is possible to distinguish healthy and cancerous mucosal tissue by determining the size distribution of epithelial cell nuclei using elastically scattered, reflected light.[1] Because the diameter of nuclei in cancerous squamous epithelial tissue are typically three to five times larger than normal nuclei (20 μm compared with 4-7 μm), the scattering cross sections for normal and cancerous tissue are markedly different. In the work of Perelman et al., the nuclear size distribution was inferred by examining the periodic dependence of the reflected light on the wavelenth of light incident on the tissue. The simplicity of this technique is predicated upon two specific properties of the tissue which they examined, the nuclei are spheroidal and the light reflected from the thin layer at the tissue surface is not completely randomized. Because many cancers arise from epithelial tissue, this approach (when coupled with endoscopic procedures) may have significant clinical application. While changes in nuclear size and density may be significant markers of dysplastic epithelium (as in the case of the Barrett's esophagus studied by Perelman et al.), changes in other parameters such as cellular index of refraction and polarization of the incident light may aid in characterizing biological tissue.[2,3]

We have a developed a technique for measuring changes in the size and relative index of refraction of spherical structures in a tenuous media; as in other forms of scattering, the technique is also sensitive to polarization changes. The essence of the method is to spatially resolve the detailed structure of the scattered light using a high numerical aperture microscope. Our method differs significantly from other techniques which seek to determine the angular dependence of the scattered light using goniometers, because we collect all the scattered light in a cone determined by the numerical aperture of the collection objective. Unlike the method of Perelman et al., only single wavelength illuminating light is required. The method has been applied to measurement of the near forward scattered light; this region is primarily governed by scattering from cell nuclei.[4]

The basis of method which we have developed (Figure 1) is to use Mie theory to determine the complete electromagnetic field scattered by a spherical particle within a homogeneous medium and to propagate the scattered field across the interface between the medium and air and subsequently through a high aperture microscope and ultimately to a detector plane. Scattered light is collected in a cone determined by the numerical aperture (NA) of the lens; for a high NA objective, this cone can be significant. Although the model is directly applicable to spherical particles of arbitrary size in a homogeneous medium, it would be possible to extend the model to more complicated situations. The detail structure of the scattering depends upon the following five parameters associated with the particle and the incident light: (1) the particle radius; (2) wavelength of illumination; (3) polarization of the incident light; (4) index of refraction of the particle and (5) index of refration of the medium. Because of the microscope objective lens, the scattering pattern also depends upon the objective's focal length and numerical aperture as well as the geometric distances associated with the particle and the imaging system.

There are essentially two steps required to determine the value for the parameters which govern the scattering. In the first step, a reference library of the scattering patterns from known particles (e.g. cell nuclei) is produced using either theoretical calculations and/or experimental measurements. In the second step, the scattering patterns from unknown structures are collected and then compared with the reference library. Neural networks have been trained with the model predictions and used to "correlate" with the experimentally measured scattering patterns.

Figure 2 shows an image of two identical, 7.0 μm diameter polystyrene spheres in index matching liquid. The image was acquired using a cooled, high resolution, CCD and Koehler illumination in a transmitted light microscope. Although the particles are the same size, their scattering is completely different because they lie at different distances from the objective lens of the microscope. Conversely, particles of different sizes, but at the same distance would also have uniquely different scattering patterns. Figure 3 shows a comparison of the experimentally measured and theoretically predicted scattering for a particle at two different distances from the

focus of the lens. In these images, the intensity is normalized with respect to the background intensity and the transverse distances are normalized with respect to the particle radius. In order to produce an accurate fit between theory and experiment, the parameters must be appropriately adjusted from their nominal value. For example, although the manufacturer provided NIST traceable measurements for the size of the spheres, the good agreement shown in Fig. 3. was only possible if the model accounted for the slight increase in the diameter of the sphere due to a swelling in the fluid.

Because of the sensitivity of the scattering pattern to the position of the scattering object from the collecting lens, one of the primary applications of this technique has been to determine the location of the scatterer in three dimensional flows where the particle is free to move. This work, however, can be extended to determine any of the parameters which effect the scattering pattern. Currently, the model and data represent transmitted light, but it would possible to use the method in reflection mode. In diffuse tissue, the image would contain both the coherent scattered light and a diffuse background and methods must be developed to separate these two contributions.

REFERENCES

1. Perelman, L. T., Backman,, V., Wallace, M., Zonios, G., Manoharan, R., Nusrat, A., Shields, S., Seiler, M., Lima, C., Hamano, T., Itzkan, I., Van Dam, J., Crawford, J. M. and Feld, M. S. “Observation of periodic fine structure in reflectance from biological tissue: a new technique for measuring nuclear size distribution”, Phys. Rev. Lett. 80, 627-630 (1998).
2. Schmitt, J.M. and Kumar, G. “Turbulent nature of refractive index variations in biological tissue”, Opt. Lett. 21, 1310-1312 (1996).
3. Hielscher, A. H., Mourant, J.R. and Bigio, I.J., “Influence of particle size and concentration on the diffuse backscattering of polarized light from tissue phantoms and biological cell suspensions,” Appl. Opt. 36, 125-135 (1997).
4. Mourant, J.R., Freyer, J.P. Hielscher, A.H., Eick,A.A. Shen, D. and Johnson, T. M. “Mechanisms of light scattering from biological cells relevant to noninvasive optical-tissue diagnostics”, *Appl. Opt. 37*, 3586-3593 (1998).
5. Khaydarov JD; Ovryn B (1996) Measurement of three dimensional velocity profiles in a thin channel flow using forward scattering particle image velocimetry (FSPIV) *Proceedings, 1996 ASME Fluids Engineering Division Summer Meeting*: 4 403-408
6. Ovryn B; Wright T; Khaydarov JD (1995) Measurement of three-dimensional velocity profiles using forward scattering particle image velocimetry (FSPIV) and neural net pattern recognition *SPIE*: 2546 112-124
7. Ovryn B; Khaydarov JD (1997) Forward scattering particle image velocimetry (FSPIV): application of Mie and imaging theory to measure 3D velocities in microscopic flows using partially coherent illumination and high aperture optics. SPIE: 2984 243-254

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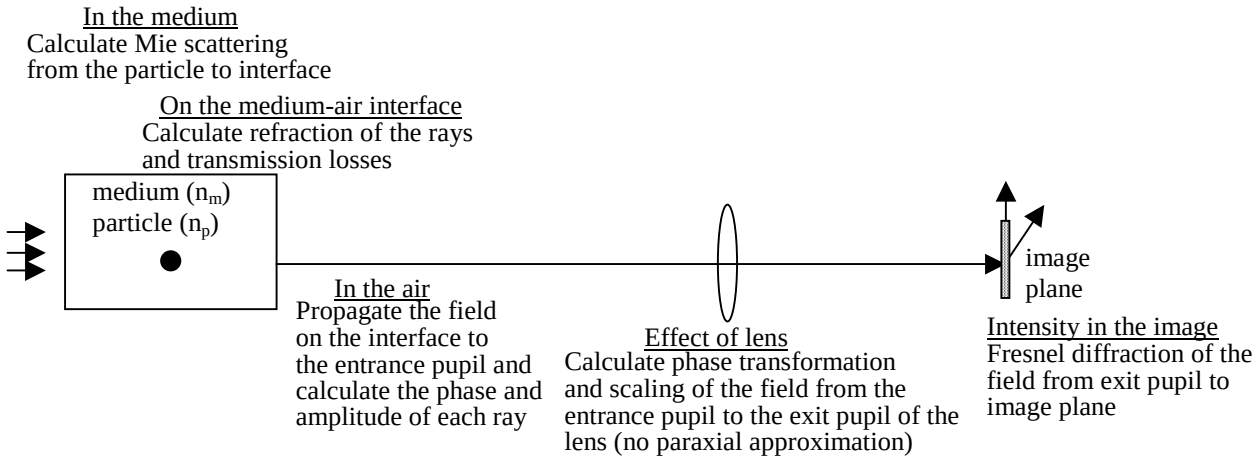

Figure 1. Model to predict the intensity in the image plane produced by the scattering from a spherical particle.

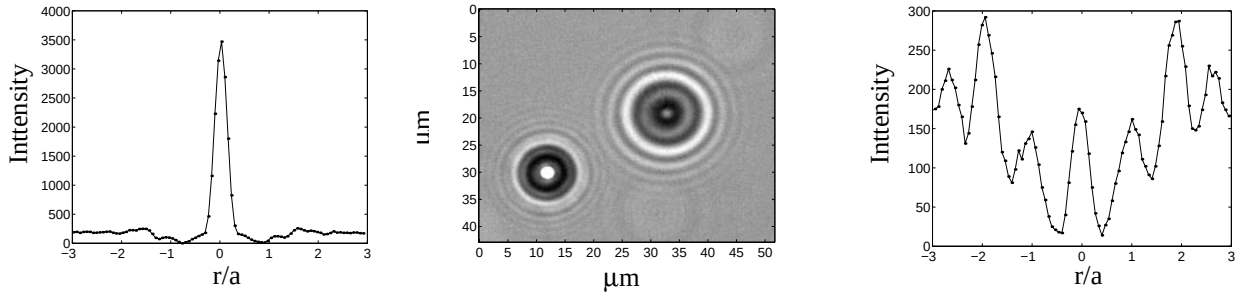

Figure 2. High density CCD image of the scattering from two 7.0 mm diameter polystyrene particles at different positions along the the optical axis. The transverse distance of each scattering pattern is normalized with respect to the particle radius, a.

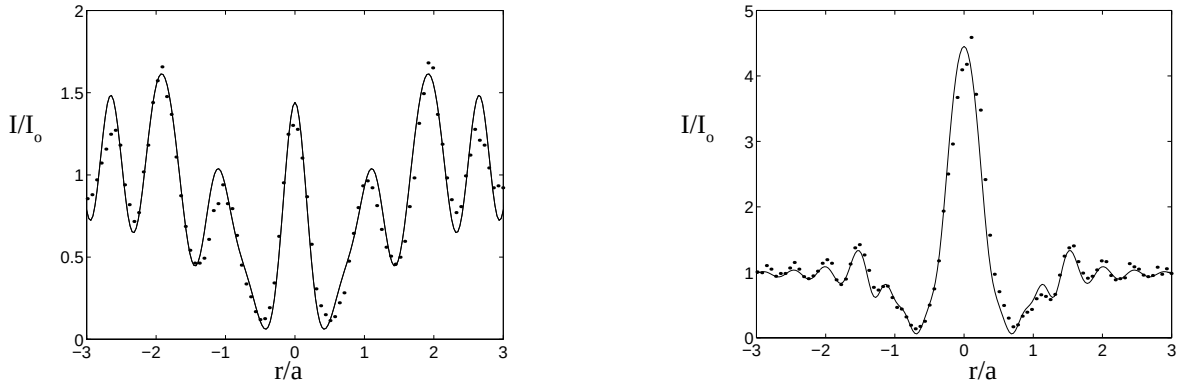

Figure 3. Comparison of measured and predicted scattering from a 7.0 μm sphere at two different locations along the optical axis.